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Revision of the 1990 working formulation for cardiac allograft rejection: the Sheffield experience

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Abstract

***Objective*—To audit the 1990 International Society for Heart and Lung Transplantation cardiac rejection criteria and to evaluate the impact on classification and clinical outcomes of a modification in which grade 2 is abolished and grades 1A and 1B are amalgamated into a single "grade 1."**

***Methods*—1652 heart biopsies were reviewed over a four year period. The initial 1348 biopsies (group 1), using the original 1990 criteria, were analysed in terms of diagnostic grade and compared with the 304 biopsies analysed with the modified scheme (group 2). Differences in grading with the 1990 scheme were compared between two groups (1.1 and 1.2) reflecting early and late experience with grading. Subsequently all the grade 2 and grade 1B biopsies were rescored in terms of the modified scheme. Clinical results in terms of actuarial patient survival at one year and freedom from 3A rejection were similarly audited.**

***Results*—The relative ratios of potentially significant rejection (grade 3A, 3B, 4) remained constant over the entire study in groups 1.1, 1.2, and 2. A 50% reduction in grade 2 biopsy reporting was noted comparing early and late parts of group 1. At subsequent review of the group 1 grade 2 biopsies, 97% could be reassigned to grades 0 or 1 in the modified scheme, with the majority of these diagnoses reflecting Quilty effect/biopsy site reactions. Two cases (3%) of the 77 grade 2 biopsies were regraded as grade 3A rejection, with both occurring within three months of transplantation. None of the grade 1B biopsies had high grade cardiac rejection on review, most of these biopsies similarly showing pronounced Quilty effect and biopsy site reactions. Actuarial survival at one year rose from 86% to 90% during the study, with freedom from 3A rejection remaining unchanged at 80%.**

***Conclusions*—The original working formulation produces consistent grading except at grade 2, which is judged to be a misnomer resulting from Quilty effect and other non-rejection phenomena. While acceptable standardisation can be achieved with the 1990 scheme, the modified scheme has advantages in that it appears to encourage clear discrimination between significant and non-significant cardiac rejection. Overall, elimination of grade 2 did not produce an increase in higher grades of cardiac rejection, and thus the value of this diagnostic grade is questioned.**

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The advent of endomyocardial biopsy in the assessment of myocardial rejection produced a plethora of histopathological grading schemes and terms to describe the changes seen. There was a clear need for standardisation within the field, leading to the introduction of the working formulation for the classification of cardiac allograft rejection, sponsored by the International Society for Heart and Lung Transplantation (ISHLT) in 1990.[1] The widespread acceptance of a clear common language for pathologists and clinicians involved with heart transplantation produced a grading consensus and permitted multicentre comparisons of treatment and outcomes. The consensus on pathologist grading was seen most clearly at the extremes on the scale reflecting absent rejection (grade 0) and potentially significant rejection (grades 3A, 3B, and 4). Quality assurance exercises have shown the main areas of difficulty to involve assessment of "mild to moderate" cardiac rejection grades.[2] A revision of the working formulation was considered in 1994, with oral presentation of the proposal at the ISHLT meeting in 1995 at San Francisco. However, the modification was never endorsed.[3] The proposed revision entailed the elimination of grade 2 cardiac rejection and the fusion of grades 1A and 1B into a single entity, grade 1, which also included biopsies showing the features that could otherwise have been graded as 2. Our centre was sufficiently impressed with the proposal that from 1995 onwards all heart biopsies have been graded in this revised format. The advantages to this modification we believe to be clearly highlighted in the following audit of biopsy grades using both the original ISHLT criteria and the modified scheme.

Methods

We searched the computer database for all heart transplant biopsies that were listed according to the biopsy grade. The initial results (group 1), based on the 1990 ISHLT criteria, were further split into two (groups 1.1 and 1.2) reflecting early experience with

Table 1 Results of the audit of biopsy grades

	1990 ISHLT		*Revised scheme*
Grade	*Group 1.1 (1992–93)*	*Group 1.2 (1994–95)*	*Group 2 (1995–96)*
0	414	265	187
1A	289	206	104
1B	13	12	—
2	73	20	—
3A	36	19	13
3B	1	0	0
4	0	0	0
Total	826	522	304

Values are numbers of biopsies in each grade.

cardiac rejection biopsy grading (1.1) and that following at least 18 months' experience (1.2), in order to assay whether the pathologists had altered practice with increasing experience with the 1990 working formulation. The final batch of results (group 2) followed introduction of the proposed revision to the 1990 formulation. The results of the biopsies are expressed in numerical form (table 1), and as a percentage total score in graphical form (fig 1).

We should emphasise that the heart transplant rate remained steady at this hospital over the study period, as did the pool of five reporting pathologists. Induction of immunosuppression until April 1993 was by antithymocyte globulin (ATG) with steroid supplementation. Thereafter few patients had ATG induction, with the steroid doses being reduced from 1 mg/kg/day with tapering schedules to a taper of 50–15 mg in decrements of 5 mg/day. Cyclosporine regimens broadly remained constant across the study period. Protocol biopsies decreased from 15 to 12 samples in the 12 months following transplantation after 1993, but clinical indications for biopsy remained constant. The biopsy frequency for the given number of patients during the three time periods and the transplantation rates assessed were broadly comparable (1.3.92 to 31.12.93, 1.1.94 to 31.5.95, and 1.6.95 to 31.8.96, comprising 44, 33, and 26 transplants). The biopsy rates for each transplant were 18.8, 15.7, and 11.7 for the three time periods, respectively.

Following initial analysis of the results, all the group 1 heart biopsies scored as grade 1B and grade 2 were retrieved from the file, reviewed, and regraded according to the modified criteria by two pathologists (SKS and AK) independently. These results are presented in table 2.

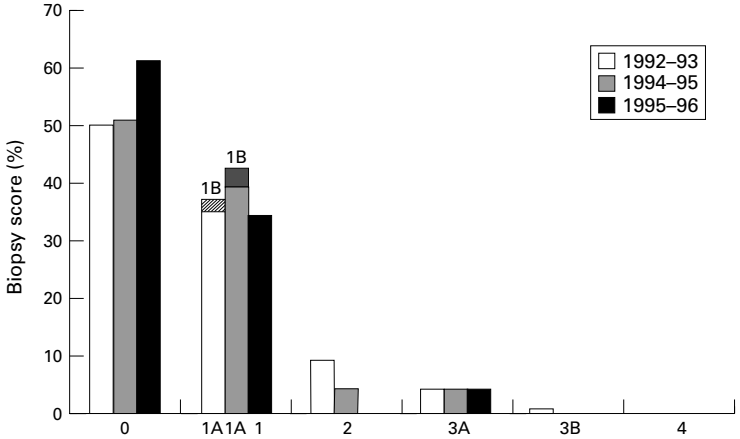

Figure 1 Histogram of the biopsy results presented in percentage terms in the three time periods of the study.

Table 2 Regrading of group 1 cases previously graded as 1B and 2

Old grade	*New grade*	*No of cases*	*Percentage*
1B (n = 22)	0	7	32
	1	15	68
	3A	0	—
	3B	0	—
	4	0	—
2 (n = 77)	0	29	38
	1	46	59
	3A	2	3
	3B	0	—
	4	0	—

Tabulated results from the regrading exercise centred on grade 1b and grade 2 biopsy samples.

Results

Analysis provides three time periods for study: early and late with the 1990 scheme, and with the revised scheme (groups 1.1, 1.2, and 2). The percentage biopsy scores (fig 1) allows clear comparison between the grades using the original 1990 working formulation and the revised scoring scheme.

We found the 1990 formulation easy to apply and noted that the extremes of the reporting spectrum were confidently graded at stable rates comparable with other United Kingdom centres. However, there was a change in the reporting of grades 1A, 1B, and 2 comparing groups 1.1 and 1.2. Most striking was the 50% reduction in reporting rate of grade 2 biopsies, with a corresponding rise in grade 1A biopsies. The rates of reporting grades 3A and above, and grades 1B and 0 biopsies were relatively stable. Overall, this difference was highly significant ($p < 0.005$, χ^2).

The modified scheme results (group 2) showed the same rate for potentially significant rejection reporting (grades 3A and above), although the loss of grade 2 and production of an amalgamated grade 1 produced changes in the percentages for grade 0 and amalgamated grade 1 cases. Grade 1 overall appeared to fall, and grade 0 to rise. This prompted the review of grade 1B and 2 biopsies, known to be most susceptible to poor grading consensus, in an attempt to dissect out the causes of the fluctuations and to assess the reliability of diagnoses given increased pathological experience.

Review of the group 1 grade 2 rejection biopsies (table 2) showed that 97% of these could be placed confidently into grades 0 and 1 in the modified scheme, with two cases (3%) representing an undercall in the degree of cardiac rejection. Of the biopsies reassigned to grades 0 and 1, the analysis showed that 68% were caused by biopsy site and Quilty effect phenomena (fig 2). Peritransplant injury, seen early in the transplant period, accounted for 8% of the misdiagnoses.

The grade 1B biopsies accounted for only a small percentage of the total samples and showed minimal variation between groups 1.1 and 1.2. None of these biopsies on review showed potentially significant cardiac rejection

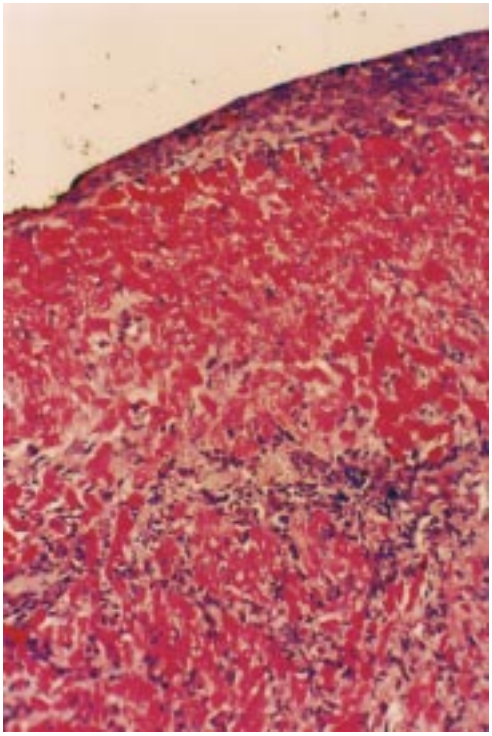

Figure 2 Quilty effect, showing a thin rind of subendocardial lymphocytes with a more diffuse infiltration in the myocardium immediately below. Inspection of the lower part of the infiltrate might provide a misdiagnosis of significant rejection if levels and associated tissues are not reviewed.

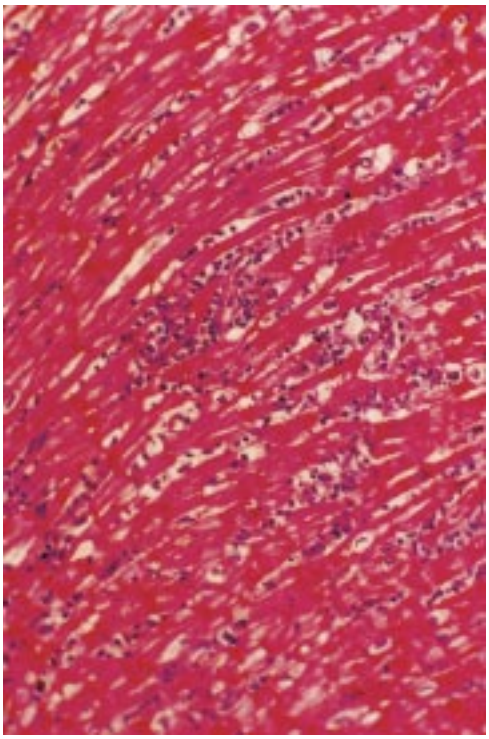

Figure 3 Endomyocardial tissue with a diffuse infiltrate of small and medium sized lymphocytes. While the appearance is suggestive of cardiac rejection, the patient was known to have post-transplant lymphoproliferative disease.

(grade 3A or above). It was possible to reassign 32% and 68% of the biopsies into grade 0 and grade 1 categories, respectively, although a single case of post-transplant lymphoproliferative disease was also revealed (fig 3).

We emphasise that the elimination of grade 2 as a diagnostic entity did not lead to any increase in potentially significant rejection rates. At the end of the time period studied, 147 heart transplants had been undertaken (male 135, female 12). Actuarial survival rates, at one year, rose during the study from 86% to 90%, with freedom from grade 3A rejection at one year being 80% throughout, except for 1992–93 where it was 35%. At the end of the study period there had been 33 deaths (primary graft failure 8, septicaemia associated 5, chronic vascular rejection 5, non-Hodgkin's lymphoma 3, ischaemic heart disease/myocardial infarction 1, other causes 11).

Discussion

The objective for the pathologist faced with a heart transplant biopsy specimen is to assess the morphological changes present and to relay meaningful information to the transplant team for optimum patient treatment. The introduction in 1990 of the ISHLT working formulation[1] permitted international standardisation of biopsy reporting for the discussion and evaluation of cardiac rejection. The numerical scale of grading cardiac rejection also allowed clear decision making in relation to treatment thresholds, and simultaneously enabled national and international statistical analyses to take place.

Our centre used the original working formulation and, having taken part in national external quality assurance programmes, we are confident that from the histopathology perspective it performs in a similar manner to other United Kingdom centres. Enhanced immunosuppression for rejection has followed a diagnosis of potentially significant rejection (grades 3A, 3B, or 4), but not lower grades. Consequently, when the modified working formulation was suggested[3] the proposal was seen as providing not only a simplification of grading protocols, but also an enhancement of decision making ability, without altering treatment standards. Our decision also reflected debate in published reports about the value of grade 2 cardiac rejection.[4 5]

Although El-Gamel *et al* have published evidence that grade 2 biopsies may progress to a higher grade lesion, particularly within the first six months after transplantation,[6] others consider that the grade 2 lesion represents chronic endocardial rejection.[7] However, Winters *et al* have found that untreated grade 2 lesions show resolution in 85% of cases, although they also noted that grade 2 biopsies within six months of transplantation were more likely to progress.[8] Milano *et al* found a similar low rate of progression.[9] An overlap has been described between the Quilty B lesion and the apparently grade 2 heart biopsy, both having similar immunohistochemical profiles.[5] We have seen that Quilty lesions, when turned through 90°, show similar focal lesions that might be mistaken for "focal moderate rejection"/grade 2 rejection.

This diagnostic dilemma was emphasised when Winters and McManus looked at inter-

observer scoring of cardiac rejection.[2] They concluded that 85% of heart biopsies could reliably be classified within the original working formulation.[1] However, the major problem was seen to reside at the level of differentiating Quilty B lesions and grade 2 rejection, with lesser problems occurring at the 1A/B:2 and 2:3A interfaces. Perhaps most reassuring with regard to the benign nature of the grade 2 lesion is the study by Gallo *et al*, in which follow up analysis of grade 2 biopsies was performed.[10] This study showed that 83.8% of these cases returned spontaneously to grade 0, 8.1% to grade 1A, 5.4% to grade 1B, and only 2.7% persisted. None worsened to grade 3A or above.

Our results indicate that the original working formulation is a useful and robust system, allowing standardisation and confident reporting. The principal result from the audit of group 1 results using the original ISHLT scheme was that the grade 2 biopsy rate fell by 50% when early and late periods using the 1990 scheme were compared. There was no accompanying rise in significant levels of rejection and, with minimal changes to clinical practice, we wondered if our original grade 2 diagnoses could be substantiated. Retesting these grade 2 biopsies revealed 97% to have been overcalls. The confounding factors are well known—Quilty effect, ischaemia, biopsy site reactions, and so on. Overall there appears to be a clear pathologist "learning curve" that we consider might have further reduced the grade 2 biopsy rate even if the modified scheme had not been introduced.

The value of grade 2 is further tested with the introduction of the modified scheme. We found that elimination of grade 2 rejection as a diagnostic entity did not lead to a rise in potentially significant cardiac rejection (grades 3A, 3B, or 4). Had grade 2 represented a significant cardiac rejection, then any potential delay in diagnosis might allow higher grades of cardiac rejection to evolve. Although we think that our grade 2 diagnoses would have been lower if we still followed the 1990 scheme, we are grateful that the modified scheme forces the pathologist to discriminate between rejection and non-rejection samples.

Nevertheless, we also acknowledge that in two cases (3%) cardiac rejection biopsies were underscored by the original scheme. Both occurred within three months of transplantation, and neither patient suffered as a result. We can only speculate on whether we would have promoted this small number of cases into significant rejection diagnoses if we had been using the modified scheme.

Within this setting of eliminating grade 2 as an entity, we were faced with the problem of "a single focus of rejection." Theoretically the modified scheme would result in a single focus of moderate cardiac rejection being regarded as grade 1 and therefore not significant, providing an adequate number of biopsy fragments were available for study.[11] We have found that such cases were rare when grade 2 "look-alikes" were discounted. Furthermore, by increasing the number of levels examined, reviewing the previous biopsy simultaneously, involving more than one pathologist in the assessment, and being aware of the full clinical details, we are able to resolve these cases without problem. We consider to be a bonus that one is not allowed to sit on the fence of grade 2, enforcing clear decision making in patient diagnosis. This change is partly the result of this centre's very close relationship between transplantation and pathology teams.

Knowledge of the potential difficulties at grade 1B also encouraged review of the 1990 scheme grade 1B cases, with similar results to those found with grade 2 samples. All these biopsies were confidently regraded as grade 0 or 1 with the modified scheme, the Quilty lesion and biopsy site reactions again featuring as the main areas of confusion. Consequently, we question whether there is a need to separate grade 1 into 1A/1B lesions. Since there appears to be no clinical or therapeutic benefit for doing so. In light of the above we feel happy with our decision in 1995 to adopt the modified scheme.

This study has also allowed us indirectly to consider our experience of the Quilty lesion. While some consider the lesion to be significantly associated with myocardial rejection,[12] others do not.[13] Alternate views have considered the Quilty lesion to represent episodic escape from immunosuppressive control by the myocardial tissue in response to fluctuating levels of immunosuppressive treatment.[14] Others have disagreed over the role of persisting Epstein-Barr virus activation with the lesions.[15 16] Our study results seem to suggest that the Quilty lesion is not a rejection phenomenon, like the lymphoid infiltrates we have seen within the kidney interstitium following renal allograft transplantation. Increased experience of Quilty lesions has also reassured us of their benign nature, and we have noted that some patients appear to develop these lesions, while others show no such propensity.

We admire the original 1990 ISHLT working formulation,[1] and have been impressed by the modified scheme in which grade 2 is abolished, grades 1A and 1B are fused, and the remaining grades are left unchanged. We are aware that some United Kingdom and European centres are similarly using this scheme or moving towards adopting the scheme (Corbishley CM, 1996; personal communication). However, given the success of the 1990 working formulation it is understood that many would be unhappy to abandon this protocol. There is good interpathologist standardisation in the original criteria, although we have documented in this study the advantages of amalgamating grades 1A and 1B and eliminating grade 2 diagnoses.

As an issue for debate, we might simultaneously argue that as treatment is initiated at our centre and other United Kingdom centres for 3A, 3B, or 4 diagnoses, the most useful approach would be to merge these three grades into a unified "high grade," perhaps called "grade 3". Although we might be charged with gross reductionism, we point to the relatively

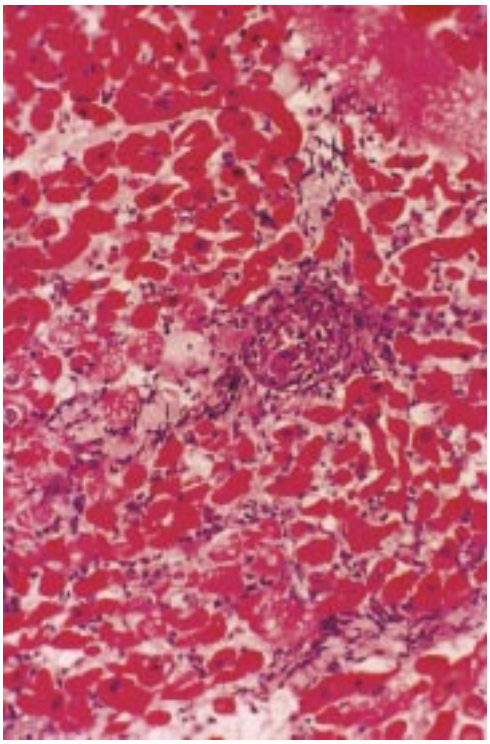

Figure 4 Endomyocardial biopsy showing a florid lymphocytic reaction centred on a small blood vessel, and suggesting vasculopathy as a manifestation of rejection. This does not fit into the ISHLT scheme, but enhanced immunosuppression could be justified. This illustrates the advantage of free text in histology reports.

low incidence of grade 3B and 4 rejection, and the availability for pathologists to record specific features in free text and code simply as "high grade rejection requiring treatment." The value of the morphological description cannot be understated, particularly as it serves as the medium for the pathologist to describe accurately cellular mechanisms seen histologically (fig 4), which may go beyond simple numerical classifications.

Finally, reverting to the modified scheme we are currently using, there is one last advantage to be emphasised. The modification allows all centres to convert their results into this new scheme retrospectively, without having to re-examine the biopsies.

In summary, we recommend this modification to the original 1990 ISHLT criteria[1] and propose a clear grading delineation between cardiac samples requiring no treatment (grade 1 and grade 0) and samples requiring treatment (grades 3A/3B/4, which could be further unified as "grade 3"). We recognise that this will not be the final word on the 1990 grading scheme, but we hope this paper will encourage debate within the field of heart transplantation.

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